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STUDIES IN EPIDEMIC POLIOMYELITIS

II. A STUDY OF THE OPSONIC ACTION OF POLIOMYELITIC SERUM ON VARIOUS ORGANISMS ISOLATED FROM FATAL CASES OF POLIOMYELITIS

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In addition to the globoid bodies isolated in conjunction with John A. Kolmer, we have isolated from the same poliomyelitic material three strains of a rather large streptococcus occurring in short chains. These strains came from the nervous tissues of two patients dying of poliomyelitis in Philadelphia during the summer of 1916, and from the nervous tissue of a monkey that contracted poliomyelitis following the injection of virus from a human being dying of poliomyelitis in West Virginia during the winter of 1917. This streptococcus was distinct from the globoid bodies, having a different appearance in culture, hanging drop, and stained specimen. The streptococci grew abundantly aerobically, while the globoid bodies required strict exclusion of oxygen. The streptococci stained differently from the globoid bodies. The globoid bodies passed readily through a Mandler filter, whereas it was only occasionally that a growth was obtained from the filtrate of a pure culture of the streptococci.

Others also have isolated cocci from cases of acute anterior poliomyelitis, notably Mathers,[1] Rosenow[2] and his associates, Nuzum and Herzog.[3] At the Philadelphia Hospital for Contagious Diseases in September, 1916, Kolmer, Brown and Freese[4] isolated many strains of Streptococci and other bacteria from the brain, cord and internal organs of fatal cases.

We have studied a number of these organisms serologically to see if serum from patients with poliomyelitis would give a specific opsonic reaction with any of them that might point toward its presence at some time in the tissues of the patient.

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[1] Jour. Infect. Dis., 1917, 20, p. 113.

[2] Jour. Am. Med. Assn., 1916, 67, p. 1202.

[3] Ibid., p. 1205.

[4] Jour. Exper. Med., 1917, 25, p. 789.

TYPES OF STRAINS EMPLOYED

Rosenow supplied us with three strains of streptococci: No. 44, "the fourth subculture of a strain from a monkey having poliomyelitis following injection of a filtrate of a virus of a monkey that was paralyzed with a filtrate of a virus."

No. 49, "a third subculture from a Berkefeld filtrate of the cyst wall of a monkey injected with a culture that was isolated from the central nervous system of a monkey paralyzed with a filtrate of a virus."

No. 707, "a strain isolated from a single colony from the spinal cord of a case of poliomyelitis in man . . . now in the ninth subculture."

Kolmer, Brown and Freese supplied us with a Streptococcus 20, isolated from the pons of a patient dying on the seventh day, in addition to many strains of staphylococci, diphtheroids and gram-negative bacilli, all obtained from poliomyelitic material.

In making the opsonic tests the method of Wright[5] was followed minutely. Cultures for the work were made on solid mediums, washed off and suspended in 1% salt solution. Human leukocytes were used, obtained in every case from the same individual. These, too, were suspended in 1% salt solution. A 1% rather than normal salt solution was used because Wright found that in this slightly hypertonic solution spontaneous phagocytosis does not take place. In making the tests equal volumes of bacterial suspension, washed blood corpuscles and serum were mixed in capillary glass tubes and incubated in a horizontal position at 37 C. for 20 minutes. Smears were made and stained with dilute carbolthionin. The bacteria ingested in the polynuclear white blood corpuscles were counted until it was observed that further counting would not change the average.

Serum was obtained from acute cases of poliomyelitis at the Philadelphia Hospital for Contagious Diseases, and from convalescents at the Wynnefield Branch of the Children's Hospital and at the Orthopedic Dispensary of the Hospital of the University of Pennsylvania. Since November, 1916, when our work was begun, there have been very few acute cases in Philadelphia and only one seen by us as early as the eighth day. Most of the convalescents were paralyzed in the summer of 1916, a few being cases of longer standing. Blood was obtained from the finger and allowed to clot. The serum was not heated and was examined the day after collection, since serum retains its opsonizing power undiminished for two or three days.

As a control, serum from the same healthy individual was carried through with every series of tests. In addition, in many series control serum was obtained from adults and children, normal so far as any history of poliomyelitis was concerned. Such serum was either tested separately, or equal volumes of a number of normal serums were mixed together and a portion of the pool thus formed tested. With the strains we used, the average number of bacteria ingested varied but slightly with the different adult serums employed. With normal children the average count was usually a little below that of the adult. The serum of normal babies less than a week old when tested with Streptococcus 20 gave an average count invariably but one-half to two-thirds of that produced by adult serum. Thus opsonin for Streptococcus 20, with which most of this work has been done, was low at birth and increased slowly with age. We have followed strictly the method of Wright, who insists that each day's work must be controlled by one or more normal serums, the average ingest of which is taken at 1.0 in computing the opsonic index.

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[5] "Technique of the Teat and Capillary Glass Tube," London, 1912.

With most of the strains tested, including No. 49, poliomyelitic serum gave results which did not justify further investigation, and early in the work we eliminated all but three strains. These three were Nos. 44 and 707, isolated by Rosenow, and 20, isolated by Kolmer, which last we have found to be identical with the streptococcus isolated by us.

TABLE 1

THE OPSONIC ACTION OF HUMAN IMMUNE POLIOMYELITIC SERUM ON STREPTOCOCCUS 20

Age	Sex	Time Since Onset	Opsonic Index	Age	Sex	Time Since Onset	Opsonic Index
3 months	M	19 weeks	0.9	2 years	M	16 weeks	1.4
6 months	F	19 weeks	1.3	2 years	F	14 weeks	1.2
7 months	M	22 weeks	1.1	2 years	F	14 weeks	1.4
8 months	F	14 weeks	0.9	2 years	M	20 months	2.0
9 months	F	19 weeks	0.6	2 years	F	5 weeks	1.7
9 months	F	9 weeks	1.0	2 years	M	12 weeks	1.8
9 months	M	8 weeks	1.1	3 years	F	19 weeks	1.6
10 months	F	19 weeks	1.4	3 years	M	2 weeks	1.3
12 months	M	3 weeks	1.3	3 years	F	19 weeks	1.5
12 months	F	?	1.5	4 years	F	17 weeks	1.8
13 months	M	15 weeks	1.0	4 years	F	4 weeks	1.1
14 months	F	18 weeks	1.4	6 years	F	17 weeks	1.1
14 months	M	7 weeks	0.8	7 years	M	20 weeks	1.3
14 months	F	18 weeks	1.5	7 years	F	7 years	1.4
16 months	M	21 weeks	1.4	13 years	F	6 years	1.3
16 months	F	21 weeks	1.1	13 years	F	10 years	1.4
16 months	M	19 weeks	1.2	15 years	F	24 weeks	1.2
18 months	F	22 weeks	1.6	16 years	F	13 years	1.5
18 months	M	21 weeks	1.4				
18 months	M	2 weeks	1.2	Controls			
19 months	M	5 weeks	1.1	7 years	F	....	1.0
20 months	F	10 days	1.7	8 years	F	....	1.0
20 months	F	?	2.2	9 years	F	....	0.8
20 months	M	6 days	1.6	Adult	F	....	1.5
21 months	F	?	1.6	Adult	F	....	1.2
2 years	M	9 weeks	1.1				
2 years	M	27 weeks	1.6	Pool of 4 adults	..	....	1.0

Average opsonic index of human immune poliomyelitic serum 1.34.

Ten serums were tested with No. 44; four of these were above normal and six below. Ten serums were tested with No. 707; eight were normal or above and two were below normal. No. 20 gave consistently high results. We tested the opsonizing power of 45 cases of acute and convalescent poliomyelitis on this streptococcus and found an index above normal in 39 instances. Two were normal. The other 4 cases were very young babies who showed an index above normal for that age. Thus this organism gave a high opsonic index in practically every case. The majority of these tests were made from 3-4 months after the onset of the disease.

In determining the opsonic index of normal children with Streptococcus 20, we tested the blood of 9 new-born babies from the Jewish Maternity Hospital, and of 20 inmates of the Home for Training in

Speech of Deaf Children. The children enter this school, which is located in the country, when 4 or 5 years of age and live there under close medical supervision until ready for high school or work. The very nature of their infirmity insures that they have associated but little with the children who go to the public schools and play on the street. In our opinion they furnish a group of children as free as could be found from mild and unrecognized attacks of poliomyelitis. As shown by the accompanying table we obtained a very low opsonic index with the nine new-born babies and a normal or low index in 18 of the 20 deaf children.

TABLE 2

THE OPSONIC ACTION OF NORMAL CHILD SERUM ON STREPTOCOCCUS 20

Age	Sex	Opsonic Index	Age	Sex	Opsonic Index
4 years	F	0.9	11 years	M	0.9
5 years	M	1.2	11 years	M	0.7
5 years	M	1.0	12 years	F	0.9
8 years	M	0.7	12 years	M	0.9
8 years	M	1.0	Babies:		
8 years	F	0.9	1 day	F	0.5
9 years	F	1.0	1 day	M	0.6
9 years	F	0.7	1 day	F	0.6
9 years	M	0.9	2 days	F	0.7
9 years	M	0.8	8 days	F	0.5
9 years	M	0.7	Pool of 4 babies	..	0.7
10 years	F	0.9			
10 years	M	0.7	Controls:		
10 years	M	0.9	Adult	M	1.0
11 years	F	0.9	Pool of 8 adults	..	0.9
11 years	F	1.2			

Average opsonic index of normal child serum 0.82.

DETAILS OF FURTHER INVESTIGATIONS

In order to investigate further any connection between Streptococcus 20 and poliomyelitis, we obtained blood from all the members of households in which one or more cases of poliomyelitis had occurred. Our object was to see if the patient gave a high index and also to see if any other member of the household might give a high index, suggesting a previous abortive attack of poliomyelitis. The serums were tested with the same technic used in our previous experiments, the one making the tests not knowing from which members of the household the blood was obtained.

Family 1 consisted of 6 individuals 2 of whom had had poliomyelitis. Tests were made with a serum heated to 55 C. for 10 minutes. No specific opsonin for Streptococcus 20 could be demonstrated in either patients. The serum of No. 5, a 16-year-old girl, contained a considerable amount of thermostable opsonin. She never had had recognized poliomyelitis.

In Family 2, the patient gave an index of 0.9, and a girl 11 years old, with no history of poliomyelitis, gave an index of 1.5.

TABLE 3

The Opsonic Action on Streptococcus 20 of Serum of Members of Households in which a Case of Acute Anterior Poliomyelitis Had Occurred

	No.	Age Years	Sex	History	Unheated Serum		Serum Heated 55 C. 10 Min.; Phagocyte Count
					Phagocyte Count	Opsonic Index	
Family 1	1	12	M	Normal	...	...	0.8
	2	4	M	A. A. P., 17 weeks*	...	...	1.0
	3	7	M	A. A. P., 18 weeks	...	...	1.3
	4	14	F	Normal	...	...	0.8
	5	18	F	Normal	...	...	0.8
	6	16	F	Normal	...	...	5.0
Family 2	1	9	M	A. A. P.	6.5	0.9	0.6
	2	11	F	Normal	11.0	1.6	1.0
	3	45	F	Normal	4.0	0.6	0.3
	4	33	F	Normal	6.0	0.9	0.6
	5	4	M	Normal	8.0	1.1	0.6
	6	2	M	Normal	2.0	0.3	0.6
		Adult	M	Normal, control	7.2	1.0	
Family 3	1	3	F	A. A. P., 30 weeks	7.0	0.9	
	2	20	F	Normal	6.0	0.7	
	3	27	M	Normal	17.0	2.1	
	4	22	F	Normal	7.0	0.9	
	5	62	M	Normal	5.0	0.6	
	6	27	M	Normal	9.0	1.1	
		Adult	M	Normal, control	8.0	1.0	
Family 4	1	26	F	Normal	13.0	1.5	
	2	4	M	Normal	10.0	1.2	
	3	2	F	A. A. P.	9.0	1.1	
	4	3	M	Normal	11.0	1.3	
	5	5	M	Normal	13.0	1.5	
	6	30	M	Normal	8.5	1.0	
		Adult	M	Normal, control	8.5	1.0	
Family 5	1	12	M	Cerebrospinal meningitis, 30 days	1.0	0.1	
	2	14	F	Cerebrospinal meningitis, 9 days	3.0	0.4	
	3	11	M	Normal	1.0	0.1	
	4	9	F	Normal	1.5	0.2	
	5	6	M	Normal	6.0	0.9	
	6	Adult	F	Normal	1.0	0.1	
	7	2	M	A. A. P., 28 days	2.0	0.3	
		Adult	M	Normal, control	7.0	1.0	

* Acute anterior poliomyelitis, 17 weeks since onset.

In Family 3, the patient gave an index of 0.9. One member of the household, an employee, gave an unusually high index, 2.1. Here again there was no history of the man having had poliomyelitis.

Five of the 6 members of Family 4 gave indexes above normal, the patient's being 1.1.

Family 5, with which are included 2 acute cases of cerebrospinal meningitis for purposes of comparison, gave very low indexes. Patient 7 was in the acute stage of poliomyelitis. The fact that this household, in which the case was an acute one, gave unusually low indexes is interesting.

We did not find the serums of the patients characterized by the presence of a large amount of opsonin. In two of the households, individuals with no history of poliomyelitis gave quite high indexes. We are unable at present to offer any explanation of these findings, having made no investigation as to how soon after onset the index begins to rise and as to how long it remains high.

In Families 1 and 3, the patients had been tested some months before, and at that time had given indexes above normal. They are included in the series of tests made with Streptococcus 20 recorded above. When examined the second time, much of the increased opsonin had disappeared. Such a gradual loss of opsonic power has been demonstrated by Mathers and Tunnicliff,[6] who used a different technic and an organism which we have shown elsewhere to be identical with Streptococcus 20.

The fact that the serum of a large percentage of patients with poliomyelitis give a high opsonic index with streptococci isolated from poliomyelitic material may not have any direct bearing on the etiology of that disease. But what it does clearly suggest, if not prove, is that these streptococci are not preagonal or post-mortem invaders. At some time before, during or after the acute febrile stage of poliomyelitis these streptococci must have been in intimate and sufficiently prolonged contact with the tissues of the patient to stimulate the production of antisubstances. Further evidence of this possibility is shown by positive complement fixation reactions observed by Kolmer and Freese[7] with the serum of a small percentage of persons in the late stages of acute anterior poliomyelitis and polyvalent antigens of streptococci.

Serum from convalescent poliomyelitic patients does not give a high opsonic index with all streptococci. The reaction, so far as we have studied, is confined to strains of rather large organisms occurring in short chains isolated from poliomyelitic material. Mathers and Tunnicliff[6] have likewise shown that the rise and fall of the opsonic power against the poliomyelitic strain is not paralleled by a similar curve when the same serum is tested with the Streptococcus pyogenes. We have found the same to be true not only with streptococci from other sources but with staphylococci, diphtheroids and bacilli of the colon group found in poliomyelitic material. These latter would seem to have established themselves post mortem.

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[6] Jour. Am. Med. Assn., 1916, 67, p. 1935.

[7] Jour. Immunol., 1916, 2, p. 327.

SUMMARY

In addition to and distinct from the globoid bodies, there is frequently present in poliomyelitic material a rather large streptococcus, occurring in short chains.

The serums of a large percentage of patients with poliomyelitis give high opsonic indexes with this streptococcus, but not with streptococci from nonpoliomyelitic sources, nor with staphylococci, diphtheroids and gram-negative bacilli obtained from poliomyelitic material.